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Acute GVHD prophylaxis plus ATLG after myeloablative allogeneic haemopoietic peripheral blood stem-cell transplantation from HLA-identical siblings in patients with acute myeloid leukaemia in remission: final results of quality of life and long-term outcome analysis of a phase 3 randomised study

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Summary

Background We previously showed that human anti-T-lymphocyte globulin (ATLG) plus ciclosporin and methotrexate given to patients with acute leukaemia in remission, having allogeneic haemopoietic stem-cell transplantation with peripheral blood stem cells from an HLA-identical sibling donor after myeloablative conditioning, significantly reduced 2-year chronic graft-versus-host disease (cGVHD) incidence and severity, without increasing disease relapse and infections, and improves cGVHD-free and relapse-free survival (cGRFS). The aim of an extended follow-up study was the assessment of long-term outcomes, which are, in this context, scarcely reported in the literature. We report unpublished data on quality of life (QoL) from the original study and the results of a follow-up extension.

Methods In the original open-label study, patients with acute myeloid and lymphoblastic leukaemia in first or subsequent remission, having sibling HLA-identical allogeneic peripheral blood stem-cell transplantation, were randomly assigned (1:1) to receive ATLG plus standard GVHD prophylaxis with ciclosporin and short-term methotrexate (ATLG group) or standard GVHD prophylaxis without ATLG (non-ATLG group). Conditioning regimens were cyclophosphamide 120 mg/kg with either total body irradiation (12 Gy) or busulfan (12·8 mg/kg intravenously or 16 mg/kg orally), with or without etoposide (30–60 mg/kg). Randomisation was stratified according to centre and disease risk. The primary endpoint was cumulative incidence of cGVHD at 2 years. The primary and secondary endpoints, excluding QoL, have been published. QoL, assessed using European Organisation for Research and Treatment of Cancer QLQ-C30 and QLQ-HDC29 questionnaires, was an unpublished secondary endpoint, which we now report here. A follow-up extension was then done, with the primary endpoint cumulative incidence of cGVHD. Enrolment has been completed for both studies. The original trial (number, NCT00678275) and follow-up extension (number, NCT03042676) are registered at ClinicalTrials.gov.

Findings In the original study, from Dec 14, 2006, to Feb 2, 2012, 161 patients were enrolled and 155 were randomly assigned to either the ATLG group (n=83) or to the non-ATLG group (n=72). In the follow-up study, which started on Feb 7, 2017, and was completed on June 30, 2017, 61 patients were included in the ATLG group and 53 were included in the non-ATLG group. Global health status showed a more favourable time course in the ATLG group compared with the non-ATLG group ($p=0\cdot02$; treatment by visit interaction). ATLG was descriptively superior to non-ATLG at 24 months for physical function (points estimate $-14\cdot8$ [95% CI $-26\cdot4$ to $-3\cdot1$]; $p=0\cdot014$) and social function ($-19\cdot1$ [$-38\cdot0$ to $-0\cdot2$]; $p=0\cdot047$), gastrointestinal side-effects ($8\cdot8$ [$2\cdot5$ – $15\cdot1$]; $p=0\cdot008$) and effect on family ($13\cdot5$ [$1\cdot2$ – $25\cdot8$]; $p=0\cdot032$). Extended follow-up (median 5·9 years [IQR 1·7–7·9]) confirmed a lower 5-year cGVHD incidence ($30\cdot0\%$ [95% CI $21\cdot4$ – $41\cdot9$] vs $69\cdot1\%$ [$59\cdot1$ – $80\cdot1$]; analysis for entire follow-up, $p<0\cdot001$), no increase in relapses ($35\cdot4\%$ [$26\cdot4$ – $47\cdot5$] vs $22\cdot5\%$ [$14\cdot6$ – $34\cdot7$]; $p=0\cdot09$), improved cGRFS ($34\cdot3\%$ [$24\cdot2$ – $44\cdot5$] vs $13\cdot9\%$ [$7\cdot1$ – $22\cdot9$]; $p=0\cdot005$), and fewer patients still in immunosuppression ($9\cdot6\%$ vs $28\cdot3\%$; $p=0\cdot017$) in the ATLG group compared with the non-ATLG group. 5-year overall survival, relapse-free survival, and non-relapse mortality did not differ significantly between groups.

Interpretation The addition of ATLG to standard GVHD prophylaxis improves the probability of surviving without disease relapse and cGVHD after myeloablative peripheral blood stem-cell transplantation from an HLA-identical sibling donor for patients with acute leukaemia in remission. Further additional benefits are better QoL and shorter immunosuppressive treatment compared with standard GVHD prophylaxis without ATLG. Therefore, in this setting, ATLG plus standard GVHD prophylaxis should be preferred over the standard GVHD prophylaxis alone.

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Research in context

Evidence before this study

We searched PubMed for articles published between Jan 1, 2000, and July 12, 2018, using the search terms “antilymphocyte globulin” AND “graft versus host disease prevention” AND “randomized” and filtered for clinical trials. We identified three prospective randomised trials on unrelated donor transplants and only one on HLA-identical sibling transplants. The first trial, published 9 years ago, showed that the addition of 60 mg/kg of anti-T-lymphocyte globulin (ATLG) to standard graft-versus-host disease (GVHD) prophylaxis (with ciclosporin and methotrexate), after a myeloablative regimen in patients transplanted from unrelated donors for their haematological malignancies, reduced the incidence of acute GVHD and chronic GVHD (cGVHD) without increasing relapse or non-relapse mortality. Overall survival and disease-free survival were not significantly different in the two groups (60 mg/kg ATLG vs standard prophylaxis). An increased incidence of cytomegalovirus and Epstein-Barr virus reactivations were recorded. The study was updated in 2017, and after a median follow up of 8.6 years, the results reported in the original study analysis were supported in the long term, showing a significant increase in the composite endpoint (severe GVHD-free and relapse-free survival [cGRFS]) from 13% to 34% after 8 years from transplant. The second trial used the same ATLG dose and schedule as the first study in patients with acute leukaemia in remission, with myelodysplastic syndromes with 10% or less bone marrow blasts, and who had a transplant from an unrelated donor after a myeloablative conditioning regimen. The addition of ATLG to standard GVHD prophylaxis (ATLG group) did not improve moderate-to-severe cGVHD-free survival, the primary endpoint, compared with standard GVHD prophylaxis (non-ATLG group). Incidence of moderate-to-severe cGVHD was significantly lower in the ATLG group than in the non-ATLG group, but progression-free survival and overall survival were lower in the non-ATLG group. Moderate-to-severe cGRFS, which was the primary endpoint of the study, was not different in the two groups. The third trial we identified focused on patients with high-risk acute myeloid leukaemia who were in remission after receiving a myeloablative transplant from an unrelated donor in a paediatric setting. Patients were randomly assigned to receive either ATLG 30 mg/kg or 15 mg/kg. Patients in the 15 mg/kg group had a significant increase in overall survival and event-free survival compared with the 30 mg/kg group due to a reduced incidence of lethal viral infections while maintaining the same effect on GVHD prevention. Finally, the only trial that focused on HLA-identical sibling transplants was published by our group in 2016. The addition of ATLG to standard GVHD prophylaxis in patients with acute leukaemia in remission, who

had peripheral blood stem-cell myeloablative transplants from HLA-identical sibling donors, significantly decreased the incidence and severity of cGVHD without a negative effect on survival, relapse, and non-relapse mortality at 2 years. Accordingly, cGRFS increased with the addition of ATLG (16.8% in the non-ATLG group vs 36.6% in the ATLG group at 2 years from transplant). No quality-of-life (QoL) data were reported in any of the randomised studies examined.

Added value of this study

This study provides two important added values. First, we report QoL data on the only randomised study that focused on sibling transplants, and show that even after adjusting for confounding factors, such as age, sex, country, and GVHD, patients in the ATLG group showed a better QoL than those in the non-ATLG group (standard GVHD prophylaxis with ciclosporin and methotrexate). In particular, global health status scoring was higher, with a more pronounced improvement over time, in the ATLG group ($p=0.02$): the treatment group marginal mean difference was 2.8 points (SEM 3.9) at day 100 and increased to 10.5 points (5.3) at 24 months, in favour of ATLG. Second, after an extended follow up (median 5.9 years [IQR 1.7–7.9]), we observed a reduction in GVHD incidence in the long term (30.0% [95% CI 21.4–41.9] in the ATLG group vs 69.1% [59.1–80.1] in the non-ATLG group at 5 years; $p<0.001$) and an improvement of 5-year cGRFS (34.3% [95% CI 24.2–44.5] vs 13.9% [7.1–22.9]; $p=0.005$) in the ATLG group compared with the non-ATLG group. Time to permanent discontinuation of immunosuppression was also significantly shortened with ATLG administration (median 6.9 months vs 19.9 months; $p=0.010$). Finally, although no improvement in overall survival was shown in the ATLG group, relapse incidence and relapse-free survival were not affected by its addition to standard GVHD prophylaxis.

Implications of all the available evidence

The results from this study will be helpful in clinical counselling of the risk of cGVHD after HLA-identical sibling peripheral blood stem-cell myeloablative transplantation for patients with acute leukaemia in remission. This risk can be reduced without a significant increase in relapse risk compared with ciclosporin and methotrexate. Additionally, the QoL is significantly improved and patients could therefore be further reassured. For these reasons, the addition of ATLG to the standard GVHD prevention with ciclosporin and methotrexate could be the preferred combination for GVHD prophylaxis in this setting. The role of ATLG in different settings of transplantation (eg, after a reduced intensity conditioning, with a different stem-cell source, or with more advanced diseases) needs to be further investigated.

Introduction

Chronic graft-versus-host disease (cGVHD) is one of the major complications after allogeneic haemopoietic stem-cell transplantation, especially when peripheral blood stem cells are used.^{1,2} Previous acute GVHD (aGVHD), age, sex mismatch, and the use of peripheral blood stem cells as stem-cell source are well recognised risk factors of cGVHD occurrence. Peripheral blood stem cells have become the most frequently used stem-cell source for allogeneic transplants,^{3,4} even though this source has been associated with a higher incidence of cGVHD in transplants with both unrelated and sibling donors.^{1,2,4} cGVHD, in particular the extensive type, has a significant effect on non-relapse mortality, morbidity, and quality of life (QoL).^{5,6} A new composite endpoint (cGVHD-free and relapse-free survival [cGRFS]) has been increasingly used to measure the outcome of the transplant procedure by assessing cGVHD-free and relapse-free survival.^{7,8} For these reasons, several attempts to improve the efficacy of GVHD prophylaxis are being made, in particular with the aim of reducing incidence and severity of cGVHD. Different preparations of rabbit anti-T-cell globulins are available and have been tested as in-vivo T-cell depletion. Human anti-T-lymphocyte globulin (ATLG) was prepared by immunisation of rabbits with a Jurkat cell line, and anti-thymocyte globulin (ATG) was obtained in rabbits against human thymocytes.

Several randomised studies have already shown that the addition of ATLG or ATG to the standard GVHD prophylaxis with a calcineurin inhibitor and methotrexate given to patients having myeloablative transplants from unrelated donors reduces both aGVHD and cGVHD incidence without increasing the relapse risk,^{9–11} with the exception of one study,¹² and improves cGRFS. However, in the case of sibling transplantation the only available randomised study¹³ showed that ATLG, given at 10 mg/kg once daily 3 days to 1 day before myeloablative sibling transplants for patients with acute leukaemia in remission, reduced the cumulative incidence of cGVHD from 68·7% to 32·2% at 2 years, without increasing relapse incidence or infectious complications. Overall survival and relapse-free survival in this study were not affected by the addition of ATLG, whereas cGRFS significantly improved from 16·8% to 36·6% at 2 years.

Here, we report unpublished data on QoL from the original study and the results of a follow-up extension, with the aim of assessing the main outcomes (cGVHD incidence, relapse risk, non-relapse mortality, overall survival, disease-free survival, and cGRFS), recording new cases of cGVHD, and assessing immunosuppression discontinuation, secondary malignancies, and occupational status after transplantation.

Methods

Study design and participants

The multicentre, open-label, phase 3 ATGFamilyStudy,¹³ was run in Italy (16 haemopoietic stem-cell transplant

programmes), Spain (six), Germany (three), and Israel (one; appendix). Patients with acute leukaemia in complete remission were randomly allocated (1:1) to receive ATLG plus the standard regimen of ciclosporin and methotrexate after a myeloablative conditioning regimen or the standard regimen alone. The myeloablative conditioning regimen consisted of cyclophosphamide (120 mg/kg) and total-body irradiation (12 Gy) or busulfan (16 mg/kg orally or 12·8 mg/kg intravenously), with or without etoposide (30–60 mg/kg). Allogeneic transplantations were performed from an HLA-identical sibling (8/8 matched) donating peripheral blood stem cells. Eligible patients were aged 18–65 years, had acute myeloid or lymphoblastic leukaemia, with adequate organ function, and were in first or subsequent complete cytological remission. All patients were admitted to the hospital for transplantation; there was no central review of diagnosis. Treatment of GVHD was given according to centre policy. Full details about the design, conduct, analysis, and results of the original study have been previously published.¹³

During the 2-year ATGFamilyStudy, QoL was assessed by means of two European Organisation for Research and Treatment of Cancer (EORTC) questionnaires—namely, the QLQ-C30, which has been developed for patients with cancer, including the setting of haemopoietic stem-cell transplantation,¹⁴ and the QLQ-HDC29 for those having intensive chemotherapy (version 3.0).¹⁵ For the long-term study, all the patients randomly assigned in the original study and who provided written informed consent were considered eligible.

The original study was approved by each competent local ethics committee, according to the Declaration of Helsinki, and both studies are registered with ClinicalTrials.gov (NCT00678275 for QoL [the original trial] and NCT03042676 for the extended follow-up).

Randomisation and masking

Patients were randomly assigned 1:1, with stratification according to centre and disease risk. The randomisation was unmasked. The randomisation code was generated by nQuery Advisor, version 6.0, with the use of a permuted, block-randomisation plan and a block size of 4. Further details have been previously published.¹³

Procedures

All patients received ciclosporin, which was started the day before transplantation (day –1) with the aim of obtaining a trough plasma concentration of 200 ng/mL or higher, in combination with methotrexate at a dose of 15 mg/m² daily on day 1 and then 10 mg/m² on days 3, 6, and 11 after transplantation. The study protocol recommended that ciclosporin should be tapered around day 120 in the absence of aGVHD (25% every 2 weeks) and discontinued at day 180, provided that there was no evidence of GVHD. ATLG (Grafalon, NEOVII Biotech, Gräfelfing, Germany), was given at a

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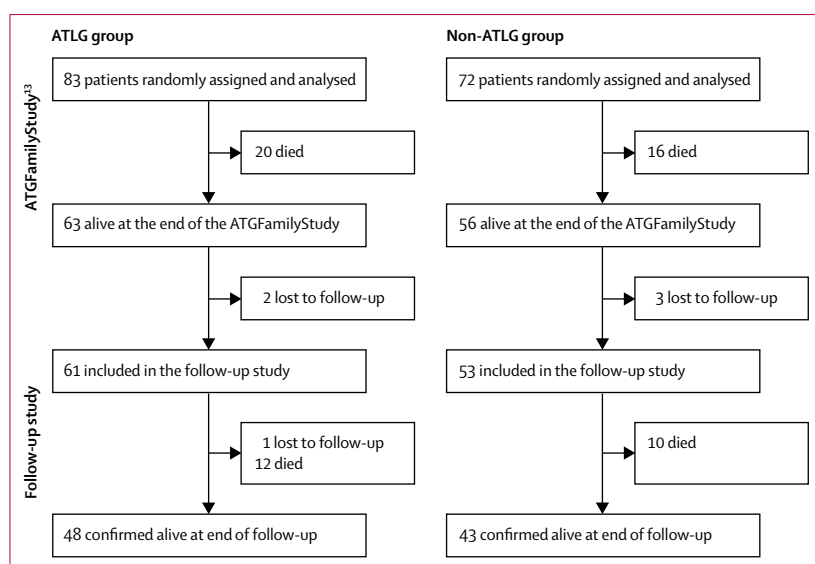


Figure 1: Trial profile
ATLG=anti-T-lymphocyte globulin.

dose of 10 mg/kg once daily from day –3 to day –1 before transplantation.

The QLQ-C30 includes one global health status scale, five functional scales (physical, role, emotional, cognitive, and social functioning), and nine symptom scales (fatigue, nausea or vomiting, pain, dyspnoea, insomnia, appetite loss, constipation, diarrhoea, and financial problems). The QLQ-HDC29 is composed of 14 symptom scales (gastrointestinal side-effects, worries or anxiety, effect on family, body image, sexuality, inpatient issues, skin problems, fever or chills, urinary frequency, aches or pain in bones, taking regular drugs, finishing things, ability to have children, and experience helping to distinguish what is important in life). For the global health status scale and the functional scales of the QLQ-C30, higher scores indicate better QoL, whereas for the symptom scales of both questionnaires, higher scores indicate more pronounced impairment of QoL. The administration of questionnaires was at admission in hospital, at 3–6 to 12–24 months after transplantation.

After the end of the main study, follow-up information was requested for all eligible patients during 2017 (February–June, 2017). For each patient the variables recorded were date, disease, and survival status at last contact, new cGVHD episodes or flares of previous cGVHD, immunosuppressive medication, occupational status, and appearance of secondary malignancies. cGVHD staging was done according to the modified Seattle criteria, as stated in the protocol, and cumulative incidence was calculated accordingly.

Outcomes

The primary endpoint of the original study was 2-year cumulative incidence of cGVHD; the predefined secondary endpoints included the incidences of engraftment,

aGVHD, non-relapse mortality, relapse-free survival, overall survival, cGRFS, infectious complications, and QoL according to the EORTC QLQ-C30 and QLQ-HDC29 questionnaires at 2 years. The results of all outcomes except QoL have been previously published.¹³ The primary objective of the follow-up study was the establishment of an electronic database including variables capturing the update of cGVHD, survival, disease relapse, immunosuppressive therapy, and the eventual recovery of working activity. Hence, similar to the original study, cGVHD incidence, overall survival, relapse-free survival, cGRFS, non-relapse mortality, relapse incidence, and immunosuppression withdrawal at 5 years were assessed as secondary outcomes and cumulative incidence of cGVHD was the primary outcome of the follow-up study. No long-term follow-ups of QoL, infections, and toxicity were done.

Statistical analysis

We assessed cGVHD, cGRFS, relapse, and non-relapse mortality by cumulative incidence analysis.¹⁶ We counted cGRFS, relapse or progression, and cGVHD of any kind as events. Competing risks were death or relapse for cGVHD, cGVHD and relapse-free death for cGRFS, non-relapse death for relapse, and relapse for non-relapse mortality. We compared treatment groups using Gray's test. We assessed overall survival and relapse-free survival by Kaplan-Meier analysis with 95% CIs based on a transformation of intervals for the log-minus-log survival function and using log-rank tests to compare the treatment groups.

We did further treatment group comparisons with the use of χ^2 tests for nominal data, Mann-Whitney *U* tests for ordinal data, and analyses of variance for interval or ratio-scale data. We used exploratory Cox multiple regression analysis for determining the confounding effect of recipient age, first versus second remission, acute lymphoblastic versus acute myeloid leukaemia, cytogenetic risk, donor or recipient sex mismatch, recipient cytomegalovirus seropositivity, type of conditioning, CD34+ cells transplanted, and grade of aGVHD and cGVHD (grade of cGVHD as time dependent variable because it was assumed that cGVHD might modify the risk of relapse and mortality) on the incidence of cGVHD, non-relapse mortality, relapse, relapse-free survival, and overall survival in addition to the effect of treatment (ATLG *vs* non-ATLG). We used forced entry for treatment and backward elimination for all other covariates.

For the investigation of QoL, we used mixed models for repeated measures and linear mixed models to analyse the time courses and the slopes of the outcomes depending on treatment group (ATLG *vs* non-ATLG), age, country, sex, and cGVHD. In particular, we did mixed models for repeated measures analyses for testing whether the independent variables and the specified interactions between them had a significant influence on the scores of the QLQ-C30 and QLQ-HDC29 scales and

their changes over time. We tested treatment group differences at particular visits using contrasts. We used linear mixed models analyses to investigate whether the groups defined by the independent variable(s) differed with regard to the slopes of the time course of the QLQ-C30 or QLQ-HDC29 scales.

For both mixed models for repeated measures and linear mixed models analyses, we fitted models using restricted maximum likelihood parameter estimation, an unstructured covariance matrix, and the Satterthwaite approximation for determining the degrees of freedom. Analyses were based on the assumption that data were missing at random—ie, missingness might have depended on observed data but not on unobserved data. To assess the robustness of the missing at random assumption, we did sensitivity analyses using multiple imputation and imposing different penalties on missing data in the ATLG group but not in the non-ATLG group. For global health status or QoL, we imposed penalties between 0·5 and 2·5 points.

All analyses were based on all patients analysed for safety and efficacy in the original 2-year study,¹³ unless otherwise noted. All p values are two-sided and are to be interpreted descriptively (the term significant is used to characterise p values $\leq 0\cdot05$ and is not intended in a confirmatory sense). For time-to-event analyses, the reported p values apply to the comparison of the cumulative incidence or survival curves across the entire follow-up. All analyses based on events occurring later than 24 months after stem-cell transplantation are to be considered post-hoc analyses. For statistical analyses we used NCSS version 10 for cumulative incidence analysis and IBM SPSS Statistics version 24 for all other analyses.

Role of the funding source

The study was an academic study. The study was supported by the European Society for Blood and Marrow Transplantation (EBMT) as an EBMT-labelled study of the Chronic Malignancies Working Party. Neovii Biotech supplied the drug for free and provided financial support to NK. Neither EBMT nor Neovii Biotech had a role in study design, data collection, data analysis and data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had the final responsibility for the decision to submit for publication.

Results

Between Dec 14, 2006, and Feb 2, 2012, 161 patients were enrolled, and six patients were excluded because of donor refusal or disease progression. 155 patients were randomly assigned to each group (n=83 in the ATLG group; n=72 in the non-ATLG group) and were included in the full analysis set. The final analysis was performed after the last patient achieved at least a 2-year observation (June 8, 2014). 119 (77%) of 155 patients were alive at the end of the study. The extension study started on

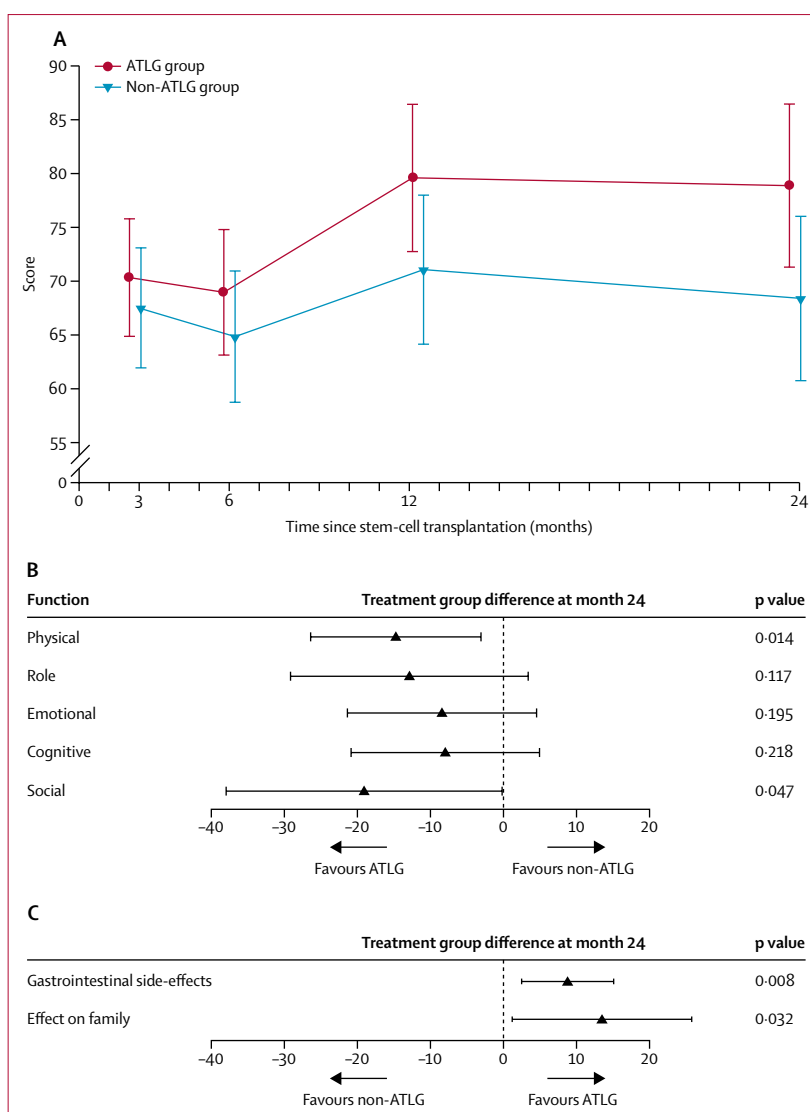


Figure 2: Quality-of-life analysis

(A) Global health status (QLQ-C30) according to treatment group. Global health status or quality-of-life time course values are expressed as marginal means with 95% CIs from MMRM analysis adjusted for baseline values (higher scores indicate a more favourable status). Patients in the ATLG group showed a greater, important improvement in global health status ($p=0\cdot02$), from a treatment group difference of 2·8 (SEM 3·9) at 3 months to 10·5 (5·3) at 24 months. (B) QLQ-C30 functional scores: differences between MMRM marginal means at 24 months after stem-cell transplantation with 95% CIs; p values are adjusted for country, age, sex, and cGVHD. (C) QLQ-HDC29 symptom scales: differences between MMRM marginal means at 24 months after stem-cell transplantation with 95% CIs; p values are adjusted for country, age, sex, and cGVHD. ATLG=anti-T-lymphocyte globulin. cGVHD=chronic graft-versus-host disease. MMRM=mixed models for repeated measures. QLQ-C30 and QLQ-HDC29=European Organisation for Research and Treatment of Cancer questionnaires.

Feb 7, 2017, and all the follow-up information was returned and completed by June 30, 2017, for all patients. 114 patients entered the follow-up study, 61 in the ATLG group and 53 in the non-ATLG group. 12 patients died in the ATLG group and 10 patients died in the non-ATLG group. One patient was lost to observation during the follow-up study and finally 48 patients in the ATLG group and 43 patients in the non-ATLG group were confirmed to be alive at the end of the follow-up on June 30, 2017

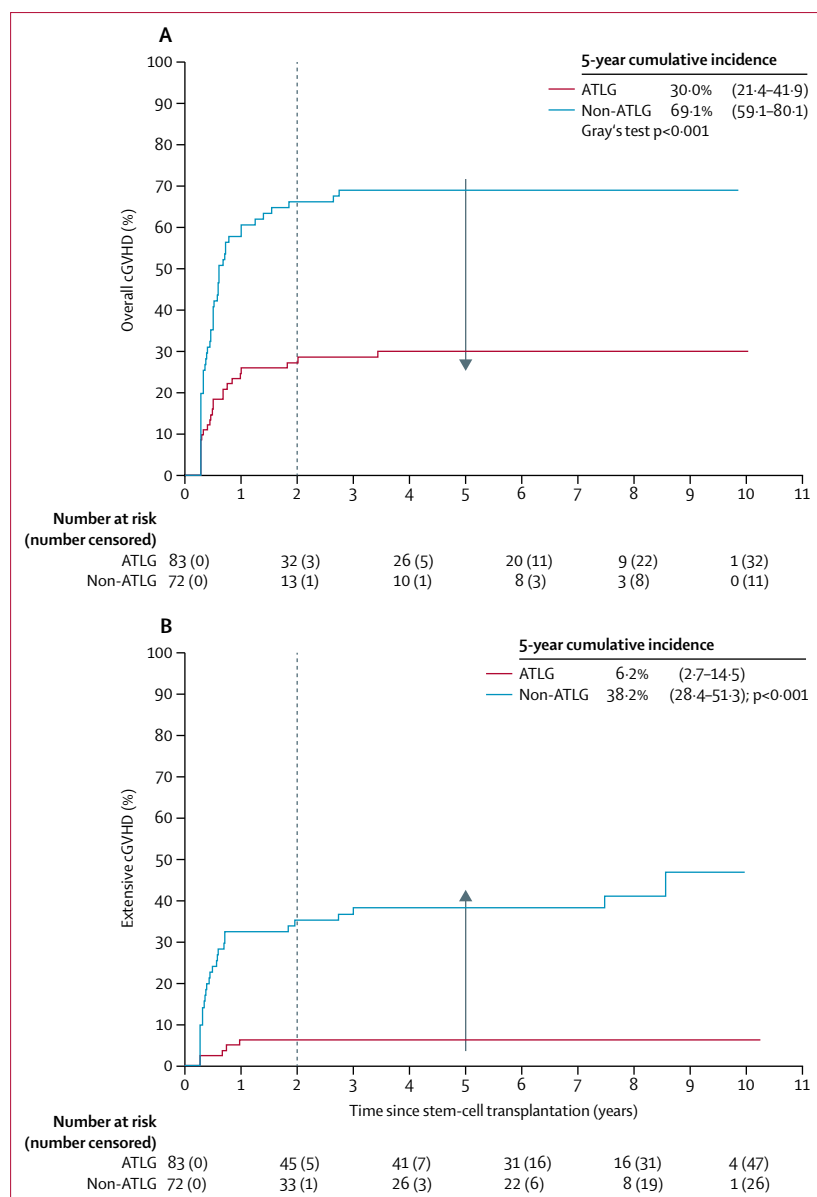


Figure 3: Cumulative incidence of cGVHD according to treatment group

(A) Overall cGVHD incidence. (B) Extensive cGVHD incidence. ATL=anti-T-lymphocyte globulin. cGVHD=chronic graft-versus-host disease. The dashed line represents the timepoint of the analysis in the original study. The large arrows represent 5-year estimates.

(figure 1). The median observation time from transplantation was 5.9 years (IQR 1.7–7.9).

According to the protocol, QoL forms were to be completed at five visits. On the assumption that all 155 patients eligible for the full analysis set would have completed the trial as scheduled, a total of 775 QoL forms would have been expected. However, 347 (45%) forms were actually retrieved, 98 (13%) forms were missing because the applicable visit was not completed (because of premature withdrawal or because the patient had missed an interim visit), and 330 (43%) forms were missing even though the patient had attended the applicable visit.

37 (24%) of 155 patients did not provide any QoL data, of whom ten were from the single participating centre in Israel, where QoL was not assessed.

Across all visits, the proportion of forms actually retrieved compared with the number that could have been completed, given the observed visit attendance, was similar between groups (50% in the ATL group and 53% in the non-ATL group). Based on actual visit attendance, the proportion of returned QoL forms decreased by visit (108 [70%] of 155 patients before stem-cell transplantation; 70 [48%] of 145 patients at 100 days, and 45 [41%] of 110 patients at 24 months after stem-cell transplantation). 41 (49%) of 83 patients in the ATL group and 43 (60%) of 72 patients in the non-ATL group provided any QoL forms after stem-cell transplantation. Centre was the variable with the closest association with availability of QoL forms: of 26 centres contributing any patients to the full analysis set, six had QoL form return rates between 75% and 100% relative to the actual visits, whereas eight had return rates between 0% and 25% (not counting the Israeli centre). Within centres, QoL form retrieval and the type of treatment received had no apparent association.

No systematic association was found between age and QoL form return. In both treatment groups women tended to be more inclined than men to complete their QoL forms (55% vs 47% of expected forms in the ATL group; 57% vs 42% in the non-ATL group). Patients with acute lymphoblastic leukaemia tended to return slightly more forms than those with acute myeloid leukaemia (57% vs 47% in the ATL group; 63% vs 49% in the non-ATL group). In the ATL group, the presence or absence of cGVHD was not associated with QoL form return (50% vs 51%), whereas a slightly higher return rate was observed for patients with cGVHD in the non-ATL group (57% vs 46%).

Most subscales of the QLQ-C30 indicated an average improvement in QoL and reduction of symptoms over time, notably in the ATL group. In a mixed models for repeated measures model controlling for country, age, sex, and cGVHD, patients in the ATL group showed significantly more pronounced improvement in global health status or QoL over time compared with patients in the non-ATL group ($p=0.02$), with a marginal mean treatment group difference of 2.8 points (SEM 3.9) at day 100 and increasing to 10.5 points (5.3) at 24 months in favour of ATL (figure 2A). Sensitivity analyses showed that superiority of ATL was still seen despite a penalty of 2.5 points on all missing data points in the ATL group, the largest penalty tested.

Beneficial effects of ATL ($p \leq 0.05$) were also observed for four of the five functional scales comparing the treatment groups across the entire trajectory of time from the start of the study to the end (24 months); whereas comparing them at a particular time (month 24), the differences were significant only for physical (points estimate -14.8 [95% CI -26.4 to -3.1]; $p=0.014$) and social (-19.1 [-38.0 to -0.2]; $p=0.047$) functioning

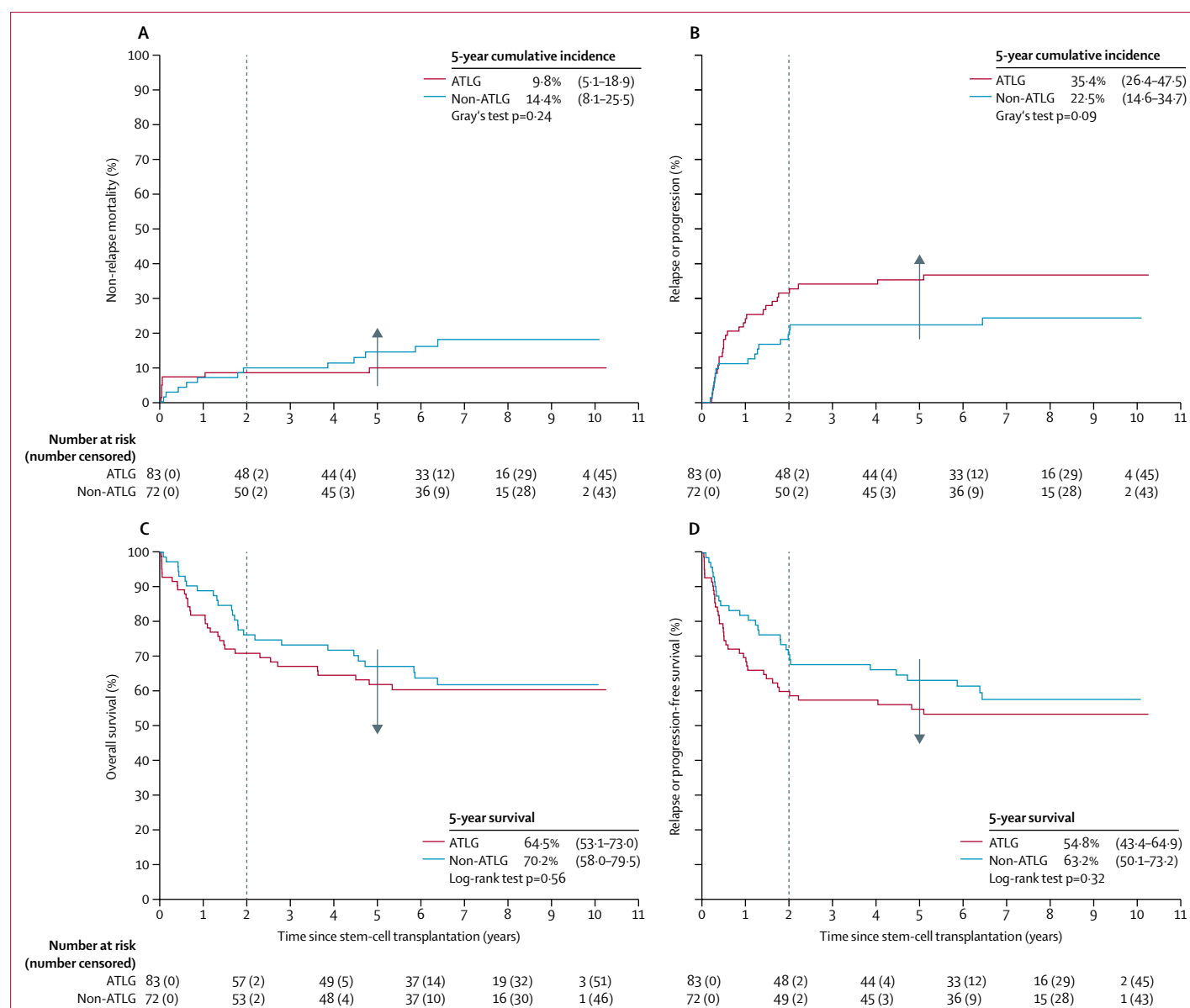


Figure 4: Non-relapse mortality (A), relapse or progression (B), overall survival (C), and relapse or progression-free survival (D) according to treatment group
 ATLG=anti-T-lymphocyte globulin. The dashed lines represent the time point of the analysis in the original study. The large arrows represent 5-year estimates.

(figure 2B). Finally for QLQ-HDC29, only the two symptom scales were reported in the analysis and they are gastrointestinal side-effects (points estimate 8.8 [95% CI 2.5–15.1]; $p=0.008$) and effect on family (13.5 [1.2–25.8]; $p=0.032$; figure 2C).

Linear mixed model analysis of QoL by country indicates that patients from Italy generally gave more favourable ratings for all functional scales and lower scores for most symptom scales than patients from Germany, whereas the time courses and slopes were similar for most scales (data not shown). Men and women showed similar QoL ratings at before and after haemopoietic stem-cell transplantation. Patients aged up

to 34 years tended to provide more favourable functional ratings, less severe symptom scores, and showed more pronounced improvements of QoL than older patients (data not shown).

In the follow-up extension study, the 5-year cumulative incidence of overall cGVHD was 30.0% (95% CI 21.4–41.9) in the ATLG group versus 69.1% (59.1–80.1; $p<0.001$) in the non-ATLG group and for extensive cGVHD was 6.2% (2.7–14.5) in the ATLG group versus 38.2% (28.4–51.3; $p<0.001$) in the non-ATLG group (figure 3A, 3B). Two new episodes of cGVHD occurred in the ATLG group beyond 2 years (both limited, one patient had already been counted as a case of cGVHD in our

	p value	Hazard ratio (95% CI)
cGVHD (-2 log likelihood=552.7; $\chi^2=41.0$; df=3; p<0.001)*		
ATLG vs non-ATLG	<0.001	0.35 (0.21-0.61)
Female donor, male recipient	0.034	1.89 (1.05-3.40)
aGvHD grade	<0.001	1.57 (1.26-1.96)
Non-relapse mortality (-2 log likelihood=223.9; $\chi^2=8.0$; df=4; p=0.091)*		
ATLG vs non-ATLG	0.583	0.79 (0.34-1.83)
Age, years	0.066	1.04 (1.00-1.08)
cGVHD	0.051	2.85 (1.00-8.14)
Remission	0.980	671270.90 (0.00-IND)
Overall survival (-2 log likelihood=410.7; $\chi^2=5.9$; df=3; p=0.117)*		
ATLG vs non-ATLG	0.491	0.81 (0.45-1.47)
Age, years	0.089	1.04 (1.00-1.06)
High vs low or intermediate cytogenetic risk	0.063	1.77 (0.97-3.24)
Relapse incidence (-2 log likelihood=336.6; $\chi^2=7.5$; df=3; p=0.0588)*		
ATLG vs non-ATLG	0.270	1.47 (0.74-2.89)
First vs second remission	0.026	0.32 (0.16-0.87)
High vs low or intermediate cytogenetic risk	0.080	1.84 (0.93-3.62)
Relapse-free survival (-2 log likelihood=475.3; $\chi^2=4.4$; df=2; p=0.112)*		
ATLG vs non-ATLG	0.662	0.89 (0.51-1.53)
High vs low or intermediate cytogenetic risk	0.039	1.79 (1.03-3.10)

aGVHD=acute graft-versus-host disease. ATLG=anti-T-lymphocyte globulin. cGVHD=chronic graft-versus-host disease. df=degrees of freedom. IND=indeterminate. *Omnibus test coefficients.

Table: Main results of multiple Cox regression models

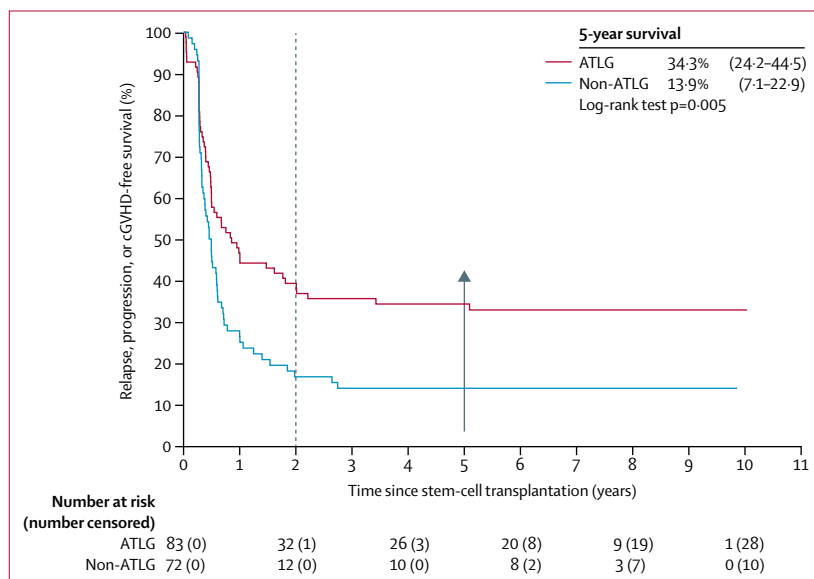


Figure 5: Chronic graft-versus-host disease and relapse-free survival according to treatment group
cGVHD=chronic graft-versus-host disease. The dashed line represents the timepoint of the analysis in the original study. The large arrow represents 5-year estimate.

original 2-year analysis¹³ but was then changed to a post-2-year case after obtaining a correction of the onset date from the centre) and three in the non-ATLG group (two limited and one extensive). Moreover, three patients in the non-ATLG group with limited cGVHD during the main study had an extensive episode during follow-up,

compared with none in the ATLG group. Five (10%) of 52 patients in the ATLG group who provided any data during follow-up were still under immunosuppression at last contact during follow-up compared with 13 (28%) of 46 patients in the non-ATLG group (p=0.017), conveying a shorter median time to permanent discontinuation of immunosuppression (6.9 months [IQR 6.1-13.2] in the ATLG group vs 19.9 months [8.3-36.2] in the non-ATLG group; p=0.010).

Cox regression analysis showed that ATLG administration (hazard ratio [HR] 0.35 [95% CI 0.21-0.61]; p<0.001), sex mismatch (female donor to male recipient; 1.89 [1.05-3.40]; p=0.034), and previous aGVHD (1.57 [1.26-1.96]; p<0.001) were significant prognostic factors for cGVHD (table). The 5-year non-relapse mortality (figure 4A) and relapse incidence (figure 4B) were not significantly different between the two groups (5-year non-relapse mortality, 9.8% [95% CI 5.1-18.9] in the ATLG group vs 14.4% [8.1-25.5] in the non-ATLG group; p=0.24; and 5-year relapse incidence, 35.4% [26.4-47.5] and 22.5% [14.6-34.7]; p=0.09).

Cox regression analysis revealed that cGVHD was the most significant predictor for non-relapse mortality (HR 2.85 [95% CI 1.00-8.14]; p=0.051), followed by recipient age (in years) (1.04 [1.00-1.08]; p=0.066), whereas for relapse incidence the most significant predictors were type of pretransplant remission (0.32 [0.16-0.87]; p=0.026) and cytogenetic risk profile (1.84 [0.93-3.62]; p=0.080). ATLG administration did not significantly affect non-relapse mortality (0.79 [0.34-1.83]; p=0.583) or relapse incidence (1.47 [0.74-2.89]; p=0.270) (table).

The 5-year overall survival (figure 4C) was 64.5% (95% CI 53.1-73.0) in the ATLG group versus 70.2% (58.0-79.5; p=0.56) in the non-ATLG group. Relapse-free survival (figure 4D) was 54.8% (43.4-64.9) in the ATLG group versus 63.2% (50.1-73.2; p=0.32) in the non-ATLG group. 12 (20%) of 61 patients who entered follow-up in the ATLG group and 10 (19%) of 53 patients in the non-ATLG group died during the follow-up period after the end of the second year after stem-cell transplantation. Cox regression analysis of the follow-up results did not support the significant association between relapse-free survival and disease type (poorer survival for acute lymphoblastic leukaemia) reported in the original study; however, we found a significant adverse effect of high cytogenetic risk (HR 1.79 [95% CI 1.03-3.10]; p=0.039; table).

The composite endpoint cGRFS (figure 5) was significantly improved by ATLG administration, with a 5-year estimate of 34.3% (95% CI 24.2-44.5) in the ATLG group versus 13.9% (7.1-22.9; p=0.005) in the non-ATLG group.

We recorded four cases of secondary malignancies; one in the ATLG group (squamous cell carcinoma of the mouth in a patient who developed limited mouth cGVHD after the end of the original study) and three in the non-ATLG group (relapse of a previous breast cancer

in a patient with limited cGVHD, and cervix and oesophageal cancers in patients affected by extensive cGVHD). Despite the significantly higher cGVHD incidence in the non-ATLG group, no appreciable difference was reported in the proportion of patients who returned to work (31 [60%] of 52 patients with valid data during follow-up in the ATL group vs 26 [56%] of 45 patients in the non-ATLG group; $p=0.583$).

Discussion

cGVHD is a major cause of late morbidity and mortality after allogeneic stem-cell transplantation.¹⁷ Additionally, cGVHD, especially in the more severe forms, is known to be associated with lower QoL.^{5,6,10} For these reasons, in the last years, much more attention has been given to QoL, and QoL measures are increasingly used as an indirect measure of efficacy in studies exploring new platforms for GVHD prophylaxis. Although mortality from cGVHD is decreasing nowadays because of better supportive care (including in particular anti-infectious therapies), deterioration of QoL is still one of the major concerns for patients having allogeneic haemopoietic stem-cell transplantation. In this study, we found that patients in the ATL group showed a higher QoL score compared with those in the non-ATLG group. In particular, global health status was significantly better in the ATL group with the treatment group difference increasing over time, as expected in a context of cGVHD, which takes time to develop and to affect organ function. Several additional QoL domains explored by QLQ-C30 and QLQ-HDC29, from physical, role, and emotional function to gastrointestinal side-effects and effect on family, favoured the ATL group too. We also found differences in QoL scoring according to country (Italian patients gave QoL scoring associated with better status than German patients). It is not clear how to explain this finding: one hypothesis is that it could be related to the habits and cultural environment, which are distinctive of each country. Cultural motivations are probably at the basis of the finding of no differences between the two groups in the proportion of patients going back to work after transplantation.

We previously showed¹³ in a randomised trial that the addition of ATL to the standard GVHD prophylaxis, after a myeloablative conditioning peripheral blood stem-cell transplant from HLA-identical sibling donor for patients with acute leukaemia in remission, reduces the incidence and the severity of cGVHD without increasing relapses or infections. The cGVHD reduction was more pronounced for the extensive type of cGVHD. Our results support, in the long term, the observation that cGVHD is prominently reduced when ATL is given. The reduction of cGVHD translated in fact into a lower proportion of patients still being under long-term immunosuppression medication at the last contact, and a shorter median time to permanent discontinuation of immunosuppression. ATL infusion was the most important prognostic factor for cGVHD

occurrence (table) and cGVHD is confirmed to be a predictive factor of non-relapse mortality, further underpinning the stringent need for effective GVHD prophylaxis to maintain low non-relapse mortality. These long-term results are consistent with those reported by Finke and colleagues¹⁸ in the setting of unrelated myeloablative conditioning transplants.

cGVHD itself, and its medications, can be expected to actively foster secondary malignancies because of reduction of cancer immune-surveillance, hence we evaluated the long-term development of secondary tumours, and found one case in the ATL group and three in the non-ATLG group—all in patients with cGVHD. The overall incidence was low and thus no significant differences were shown, but no additional risks for neoplasms can be attributed to ATL.

As an immunosuppressive drug, ATL showed that it can increase infections and Epstein-Barr virus-associated lymphoproliferative disorders, at least in an unrelated setting in which higher doses are used. We previously showed¹³ that at the dose of 30 mg/kg in sibling myeloablative conditioning transplants, ATL did not increase the incidence of Epstein-Barr virus-associated lymphoproliferative disorders, of cytomegalovirus, or of bacterial and fungal infections.

Although cGVHD reduction by ATL is an obvious and recurrent effect that can be replicated in several settings with high dose range variation, the effect on relapse is more controversial. In myeloablative conditioning unrelated transplants, two randomised studies^{11,12} that used ATL at the same doses showed a similar effect on reduction of aGVHD and cGVHD, but it had a discordant effect on relapse. Other randomised studies with different doses and timing of ATL did not find an increase in relapses. In the setting of sibling myeloablative conditioning transplants, a randomised study,¹³ and several retrospective analyses,¹⁹ were all in agreement about the absence of influence on relapses. Finally, in the setting of reduced intensity conditioning, caution for in-vivo T-cell depletion was suggested by an International Bone Marrow Transplant Registry study²⁰ especially for advanced diseases, whereas for acute myeloid leukaemia in first remission, ATG or ATL administration was not significantly associated with a higher relapse incidence.²¹ Although most relapses after transplants for acute leukaemia occur within 2 years, we did not find a significant increase in relapses at 2 years or at 5 years in our study. However, in the abovementioned studies the most used polyclonal serum was antithymocyte globulin (Thymoglobulin, Sanofi, Lyon, France) and not ATL (Grafalon, NEOVII, Grafelfing, Germany), which is the one used in the present study. The two products differ in the manufacturing process, pulsed antigens, and antibody specificities; for this reason, conclusions drawn from studies with one preparation cannot be extrapolated to those with the other one. Relapse depends on several factors, such as disease phase, intensity of conditioning

regimen, type of transplant, and GVHD prophylaxis. ATLG is a polyclonal serum exerting its activity by depleting various cells of the immune system, such as T and B lymphocytes, by several mechanisms, including complement mediated lysis, antibody-dependent cell-mediated cytotoxicity, increasing apoptosis, modulation of the function of cells involved in the migration phase of inflammation, interference with dendritic cells feature, and function and induction of T-cell regulatory cells.^{22,23}

The number of lymphocytes might be crucial for the determination of the effectively active dose of ATLG, since these cells represent the major target of the drug. A pharmacokinetic or pharmacodynamic model suggests that the area under the curve of ATG is predictive of transplant outcome influencing the immune reconstitution, GVHD, and relapse probability.²⁴ The pharmacokinetics can be predicted by the number of lymphocytes at the time of first ATG infusion, and the lymphocyte count has been proposed as criterion for ATG dosing instead of the standard per kg.²⁵ A post-hoc analysis of the study reported by Soiffer and colleagues¹² found a significant association between lymphocyte count and outcome, and further studies are needed to support this intriguing hypothesis.

Unfortunately, we could not investigate any correlation between the number of lymphocytes and the outcome because the data were not captured in the case report forms of the ATGFamilyStudy. In the absence of a prospective, controlled study validating the pharmacokinetic or pharmacodynamic approach, a general tendency to decrease the dose of ATLG is seen. In a randomised paediatric study,²⁶ a lower dose of ATLG (15 mg/kg vs 30 mg/kg) resulted in an improved event-free survival with maintenance of the anti-GVHD effect. In this analysis, with the limitation of the power of the study, relapse was not associated with ATLG infusion even with a longer observation period, whereas anti-GVHD activity was clearly maintained. Several reasons explaining these findings could be hypothesised, such as the dose used in this trial, which was half of that used in the randomised studies on unrelated transplants, the preparing regimen, which was myeloablative, and the haematological disease in remission. Finally, a potential antileukaemic effect of ATLG cannot be excluded.^{27,28}

The Cox regression analysis supports, as in our previous trial report,¹³ that the use of ATLG does not affect relapse risk, whereas it was clearly linked to the type of disease remission and to the disease risk—ie, the cytogenetic profile. In particular, the evidence that ATLG was not associated with increased relapse risk, specifically in higher risk subgroups, supports the use of ATLG in addition to the standard GVHD prophylaxis with ciclosporin and methotrexate as a new standard for GVHD prophylaxis in this transplantation setting.

Overall survival and relapse-free survival were not different in the two groups. The reasons why the conspicuous decrease in the occurrence of cGVHD,

especially the extensive form, without a significant increase of relapse did not translate into a survival advantage cannot be fully explained. The long-lasting benefit of ATLG is further supported by the improved cGRFS we found in the original report and in the extended follow-up, reassuring patients about the actual success of transplantation.

This study has some limitations: first, in this analysis we have reported cGVHD incidence according to the Seattle modified classification, as stated in the protocol, because at the time of the study planning, the National Institutes of Health classification,²⁹ although already published, was not yet standard for most transplant programmes in Europe. Second, the interpretation of the QoL is limited by the open-label design of the study, which could be particularly important for a patient-reported outcome, and is also limited by a substantial amount of missing data, which could introduce bias and could thus interfere with the robustness of the QoL findings. The results suggest, however, that missingness of QoL data was mainly associated with the observation of a clinical endpoint (death or relapse or progression), which caused premature study termination, or with deficiencies in patient management and QoL form retrieval at some of the participating centres, which occurred independently of the treatment received. Although the reasons for missingness could have inflicted some biases on the overall levels of the QoL scores produced from longitudinal modelling in mixed linear model analyses, it appears to be extremely improbable that they might have biased the comparisons between the treatment groups. Moreover, sensitivity analyses showed that the deviations from the missing at random assumptions underlying mixed linear modelling might not have been substantial. Third, it is important to note that all analyses based on data acquired more than 24 months after stem-cell transplantation had not been predefined in the original protocol of the 2-year study¹³ and are thus to be considered post-hoc analyses.

In conclusion, long-term observation of patients with acute leukaemia in remission given ATLG in the setting of HLA-identical sibling myeloablative peripheral blood stem-cell transplants supports the benefit of the addition of ATLG in terms of improved cGRFS and lower cGVHD incidence and severity, without increase of leukaemia relapse. Consequently, QoL was improved and over the years, the need for immunosuppression was reduced. In our opinion, these findings support consideration of the addition of ATLG to ciclosporin and methotrexate as a new standard for GVHD prophylaxis in this setting, and eventually comparison of it with the new GVHD prophylaxis platforms that are rapidly evolving—in particular the post-transplant cyclophosphamide.³⁰

Contributors

FB, CSo, and NK designed the study, discussed the results of the analysis, and wrote the paper. FB wrote the first draft of the manuscript. CW, MS, FP, FZ, AN, CSe, AMR, GMe, WB, PH, AS, AMC, MC, SG, JF, RS, CF, JS, DR, EB, GMi, FB, MH, DP, MJ, ET, FN, FA, and TR enrolled patients, collected data, and edited the manuscript. AV did statistical analysis. All authors approved the final version of the manuscript.

Declaration of interests

FB and NK received speaker fees and served on the advisory boards for NEOVII Biotech. NK received a research grant from NEOVII Biotech during the conduct of the study. All other authors declare no competing interests.

Data sharing

The study did not foresee a data sharing plan. Individual participant data that underlie the results reported in this Article, after deidentification and a dictionary defining each field in the set, will be made available only after approval of the proposal from the Study Committee (FB, CS, and NK) for meta-analyses. The signature of data access agreement will be requested after the approval. The application should be addressed to n.kroeger@uke.de beginning 3 months and ending 5 years after the manuscript publication. Study protocol and informed consent can be requested, during the same time frame, from francesca.bonifazi@unibo.it.

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References

- 1 Stem Cell Trialists' Collaborative Group. Allogeneic peripheral blood stem-cell compared with bone marrow transplantation in the management of hematologic malignancies: an individual patient data meta-analysis of nine randomized trials. *J Clin Oncol* 2005; **23**: 5074–87.
- 2 Anasetti C, Logan BR, Lee SJ, et al. Peripheral-blood stem cells versus bone marrow from unrelated donors. *N Engl J Med* 2012; **367**: 1487–96.
- 3 Passweg JR, Baldomero H, Peters C, et al. Hematopoietic SCT in Europe: data and trends in 2012 with special consideration of pediatric transplantation. *Bone Marrow Transplant* 2014; **49**: 744–50.
- 4 Arai S, Arora M, Wang T, et al. Increasing incidence of chronic graft-versus-host disease in allogeneic transplantation: a report from the Center for International Blood and Marrow Transplant Research. *Biol Blood Marrow Transplant* 2015; **21**: 266–74.
- 5 Lee SJ, Kim HT, Ho VT, et al. Quality of life associated with acute and chronic graft-versus-host disease. *Bone Marrow Transplant* 2006; **38**: 305–310.
- 6 Pidala J, Kurland B, Chai X, et al. Patient-reported quality of life is associated with severity of chronic graft-versus-host disease as measured by NIH criteria: report on baseline data from the Chronic graft-vs-host disease Consortium. *Blood* 2011; **117**: 4651–57.
- 7 Holtan SG, DeFor TE, Lazaryan A, et al. Composite end point of graft-versus-host disease-free, relapse-free survival after allogeneic hematopoietic cell transplantation. *Blood* 2015; **125**: 1333–38.
- 8 Solomon SR, Sizemore C, Zhang X, et al. Current graft-versus-host disease-free, relapse-free survival: a dynamic endpoint to better define efficacy after allogeneic transplant. *Biol Blood Marrow Transplant* 2017; **23**: 1208–14.
- 9 Bacigalupo A, Lamparelli T, Bruzzi P, et al. Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). *Blood* 2001; **98**: 2942–47.
- 10 Walker I, Panzarella T, Couban S, et al. Pretreatment with anti-thymocyte globulin versus no anti-thymocyte globulin in patients with hematological malignancies undergoing haematopoietic cell transplantation from unrelated donors: a randomised, controlled, open-label, phase 3, multicentre trial. *Lancet Oncol* 2016; **17**: 164–73.
- 11 Finke J, Bethge WA, Schmoor C, et al. Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. *Lancet Oncol* 2009; **10**: 855–64.
- 12 Soiffer RJ, Kim HT, McGuirk J, et al. Prospective, randomized, double-blind, phase 3 clinical trial of anti-T-lymphocyte globulin to assess impact on chronic graft-versus-host disease-free survival in patients undergoing HLA-matched unrelated myeloablative hematopoietic cell transplantation. *J Clin Oncol* 2017; **35**: 4003–11.
- 13 Kröger N, Solano C, Wolschke C, et al. Antilymphocyte globulin for prevention of chronic graft-versus-host disease. *N Engl J Med* 2016; **374**: 43–53.
- 14 Grulke N, Albani C, Bailer H. Quality of life in patients before and after haematopoietic stem cell transplantation measured with the European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Core Questionnaire QLQ-C30. *Bone Marrow Transplant* 2012; **47**: 473–82.
- 15 Velikova G, Weis J, Hjermstad MJ, et al. EORTC Quality of Life Group. The EORTC QLQ-HDC29: a supplementary module assessing the quality of life during and after high-dose chemotherapy and stem cell transplantation. *Eur J Cancer* 2007; **43**: 87–94.
- 16 Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc* 1999; **94**: 496–509.
- 17 Martin PJ, Counts GW Jr, Appelbaum FR, et al. Life expectancy in patients surviving more than 5 years after hematopoietic cell transplantation. *J Clin Oncol* 2010; **28**: 1011–16.
- 18 Finke J, Schmoor C, Bethge WA, et al. Long-term outcomes after standard graft-versus-host disease prophylaxis with or without anti-human-T-lymphocyte immunoglobulin in haematopoietic cell transplantation from matched unrelated donors: final results of a randomised controlled trial. *Lancet Haematol* 2017; **4**: e293–301.
- 19 Rubio MT, D'Aveni-Piney M, Labopin M, et al. Impact of in vivo T cell depletion in HLA-identical allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission conditioned with a fludarabine iv-busulfan myeloablative regimen: a report from the EBMT Acute Leukemia Working Party. *J Hematol Oncol* 2017; **10**: 31.
- 20 Soiffer RJ, Lerademacher J, Ho V, et al. Impact of immune modulation with anti-T-cell antibodies on the outcome of reduced-intensity allogeneic hematopoietic stem cell transplantation for hematologic malignancies. *Blood* 2011; **117**: 6963–70.
- 21 Baron F, Labopin M, Blaise D, et al. Impact of in vivo T-cell depletion on outcome of AML patients in first CR given peripheral blood stem cells and reduced-intensity conditioning allo-SCT from a HLA-identical sibling donor: a report from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant* 2014; **49**: 389–96.
- 22 Roeder T, Katzfuß M, Matos C, et al. Antithymocyte globulin induces a tolerogenic phenotype in human dendritic cells. *Int J Mol Sci* 2016; **17**: 2081.
- 23 Shimoni O, Nagler A, Gellman YN, et al. Anti-T lymphocyte globulin (ATG) induces generation of regulatory T cells, at least part of them express activated CD44. *J Clin Immunol* 2012; **32**: 173–88.
- 24 Admirall R, Nierkens C, Moniek A, et al. Association between anti-thymocyte globulin exposure and survival outcomes in adult unrelated haematopoietic cell transplantation: a retrospective, pharmacodynamic cohort analysis. *Lancet Haematol* 2017; **4**: e183–91.
- 25 Storek J. Anti-thymocyte globulin dosing—per kg or per lymphocyte? *Lancet Haematol* 2017; **4**: e154–55.
- 26 Locatelli F, Bernardo ME, Bertina A, et al. Efficacy of two different doses of rabbit anti-T-lymphocyte globulin to prevent graft-versus-host disease in children with hematological malignancies transplanted from an unrelated donor: a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol* 2017; **18**: 1126–36.
- 27 Dabas R, Lee R, Servito MT, et al. Antithymocyte globulin at clinically relevant concentrations kills leukemic blasts. *Biol Blood Marrow Transplant* 2016; **22**: 815–24.
- 28 Westphal S, Brinkmann H, Kalupa M, Wilke A, Seitz-Merwald I, Penack O. Anti-tumor effects of anti-T-cell globulin. *Exp Hematol* 2014; **42**: 875–82.
- 29 Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease. I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant* 2005; **11**: 945–56.
- 30 Kanakry CG, Bolaños-Meade J, Kasamon YL, et al. Low immunosuppressive burden after HLA-matched related or unrelated BMT using posttransplantation cyclophosphamide. *Blood* 2017; **129**: 1389–93.